Periodontal and Bone Regeneration Using Osteoinductive and Osteopromotive Biomaterials

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CHAPTER 3

Bone Morphogenetic Protein-2 Incorporated Beta-Tricalcium Phosphate Enhanced Bone Regeneration of Critical-Sized Bone Defects in Rats

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Abstract

Although preclinical and clinical studies have shown the benefits of bone morphogenetic protein-2 (BMP-2) in bone regeneration, there are increasing concerns about its side effects. These are mainly due to the high dosage of BMP-2 which is necessary to obtain the desired clinical results. Previously our group has developed a novel controlled-release delivery system; the biomimetic calcium phosphate coating incorporated with BMP-2. It can be used at much lower concentrations of BMP-2 than those used in the commercially available product and still produce similar biological effects. In this study, we made a primarily biological evaluation of BMP-2 incorporated beta-tricalcium phosphate (β-TCP) for bone regeneration in critical-sized bone defects. Critical-sized calvarial defects were created in rats. They were divided into four groups as follows: (1) empty defects (control), (2) defects filled with β-TCP, (3) defects filled with BMP-2 incorporated β-TCP, (4) defects filled with autologous bone. Eight weeks after the operation, the efficiency of the materials was evaluated using histology and histomorphometry. Moreover, the safety of the materials was evaluated using routine blood examination, blood biochemistry examination and histopathological examination of viscera. BMP-2 incorporated β-TCP demonstrated the efficiency of bone regeneration that was comparable with autologous bone, with the highest levels of new bone formation (38.3±8.4 mm3 versus 30.1±9.9 mm3, $p < 0.05$). All lab index of blood in these four groups were within the normal range. Moreover, no change related to the treatment was noted in the histopathological examination of viscera. The results from the present study demonstrated that BMP-2 incorporated β-TCP could be a promising substitute for autologous bone used for bone regeneration. Future clinical trials and preclinical trials with large animal models are necessary to investigate the safety and efficacy of BMP-2 incorporated β-TCP.

Keywords: bone morphogenetic protein-2, controlled-release, critical-sized bone defects, biological evaluation

Introduction

For decades, researchers have been developing ideal bone substitutes that compare favorably with autologous bone, which is regarded as the gold standard for bone regeneration [1]. Calcium phosphate bioceramics, including beta-tricalcium phosphate (β-TCP), hydroxyapatite (HA) et al. are the most attractive synthetic bone substitutes because of their excellent osteoconductivity [2-5]. However, they lack osteoinductivity and osteogenicity. These disadvantages have hindered their clinical application [6].

To endow these bone substitutes with osteoinductivity and a superior capability of bone formation, bone morphogenetic proteins (BMPs) particularly BMP-2, have been extensively studied and they have shown satisfactory results in repairing bone fractures and defects [7-8]. Although BMP-2 has been approved for use in orthopedics and oral/maxillofacial surgery, there are increasing concerns about its side effects, resulting from the high dosage of BMP-2 [9-10]. A great challenge is to reduce the dosage of BMP-2 without decreasing its osteogenic capability.

Previously our group has developed a novel controlled-release delivery system, the biomimetic calcium phosphate coating [11-13]. The BMP-2 can be incorporated into the crystalline latticework of the coating and released gradually and steadily [11, 14-15]. In comparison with the commercially available product with its high dose of BMP-2, this delivery system with a low pharmacological level of BMP-2 has proven to achieve a satisfactory bone regeneration [16-17].

In this study, we prepared a biomimetic calcium phosphate coating based bone substitute, BMP-2 incorporated β-TCP. We hypothesized that it could function both safely and efficiently as the autologous bone when used for bone regeneration. We made primarily biological evaluation by exploring the systemic biocompatibility and safety, and the local osteogenic capability in critical-sized bone defects of rats.

Material and methods

Preparation and characterization of the BMP-2 incorporated β-TCP.

The BMP-2 incorporated β-TCP was produced by the well-established biomimetic coating protocol[11]. In brief, amorphous calcium phosphate coating

was deposited on the surface of β-TCP (in size of 0.25-1mm, in macropores of approximately 100 μm and in porosity of about 70%, CAM Bioceramics B.V., Leiden, Netherlands), by 5-fold-concentrated simulated body fluid (5×SBF, 684 mM NaCl, 13.5 mM KCl, 9 mM $CaCl_2.2H_2O$, 2.1 mM $Na_2HPO_4.2H_2O$, 59.5 mM $NaHCO_3$, 5 mM $MgCl_2.6H_2O$) with a solution volume to granule weight ratio of 1L 5×SBF per 1g β-TCP granules for 24 h at 37°C. Subsequently, crystalline calcium phosphate and BMP-2 (50 μg, Medtronic, Minneapolis, USA) were coprecipitated on the particles (0.2 g) by supersaturated calcium phosphate solution (100 ml, 40 mM HCl, 137 mM NaCl, 4 mM $CaCl_2.2H_2O$, 2 mM $Na_2HPO_4.2H_2O$, 50 mM TRIS, PH: 7.4 ± 0.05) for 48 h at 37°C. The entire procedure was conducted under sterile conditions.

The surface characteristic of BMP-2 incorporated β-TCP was monitored using a scanning electron microscope. The loading of BMP-2 was determined by ELISA after the coating was dissolved in 0.5 M ethylenediaminetetraacetic acid (EDTA, pH 8.0).

Evaluation in vivo.

Critical-sized calvarial defects 8 mm in diameter were created in rats, and they were divided into four groups (n = 7 per group) as follows: (1) empty defects (control), (2) defects filled with β-TCP (0.1g per defect), (3) defects filled with BMP-2 incorporated β-TCP (0.1g per defect), (4) defects filled with autologous bone. Autologous bone was harvested from the drilled skull, cut into portions and then grafted in situ. After bone grafting, a barrier membrane (12.5 × 12.5 mm, Bio-Gide®, Geistlich Biomaterials, Switzerland) was used to cover the defects. Then, the skin was repositioned and sutured.

Eight weeks after the operation, the animals were euthanatized, and their blood was collected from the abdominal aorta for routine examination and biochemical examination. After gross necropsy with macroscopic observation, the visceral organs and the calvarial samples were collected and then embedded with paraffin and resin respectively for microscopic observation. According to the systematic random sampling protocol, the calvarial samples were cut into slices in a thickness of 600 μm with an interval of 1 mm. After being ground and polished to the thickness of 50 μm using the bone grinding slice technique, the slices were stained with McNeal's tetrachrome, basic fuchsine and toluidine blue O. The bone volume, bone density, fibrous tissue density, and residual material density

were measured for histomorphometric analysis using the point-counting technique. Seven slices of each sample were used for the quantitative histomorphometric analysis.

Statistical analysis was performed by one-way ANOVA and SNK t-test using SPSS software, and statistically significant values were adopted as $p<0.05$.

Results and discussion

Under scanning electron microscopy, a crystalline outer layer with an interlacing network of the plate-like and needle-like crystal was found in both the incorporated β-TCP without BMP-2(Fig 1B) and the BMP-2 incorporated β-TCP (Fig 1C). Moreover, the surface appearance and crystal structure of these coating were almost identical to that of coating we had developed previously. The ELISA result showed that the loading of BMP-2 in per gram of BMP-2 incorporated β-TCP was 58.35 ± 3.22 μg with an incorporation rate of 23.3 ± 1.3%.

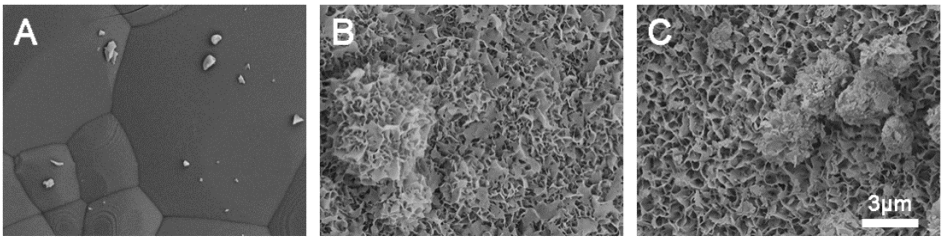

Figure 1. SEM images of β-TCP (A), incorporated β-TCP without BMP-2 (B), and BMP-2 incorporated β-TCP (C).

No local or systematic infection following the bone grafting was observed throughout the test period. The data from hematology and the biochemistry examination (Table 1) showed no difference in the number of white blood cells (WBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, creatinine (CREA) and urea between the control group and the BMP-2 incorporated β-TCP group. Although some results were statistically significant when the defects were filled with β-TCP or with autologous bone compared with the empty defects, all parameters were within the normal range. The results of histopathology showed that no changes related to the treatment were noted in the visceral organs examined which included liver, kidney, spleen and adrenal gland (data not shown). In summary, the local applied BMP-2 incorporated β-TCP demonstrated favorable systemic biocompatibility and safety.

	Control	β-TCP	BMP-2 incorporated β-TCP	Autologous bone
WBC ($\times 10^9$/L)	6.04±0.83	9.74±2.88*	5.72±1.78	11.00±4.50*
ALT (U/L)	55.86±30.41	61.13±13.12	59.99±25.28	41.11±6.85
AST (U/L)	155.77±49.13	178.26±29.96	186.16±38.28	123.44±27.55
AST/ALT	3.01±0.65	2.99±0.48	3.39±0.99	3.16±1.14
CREA (μmol/L)	69.81±15.67	50.33±6.87*	66.51±10.91	63.23±3.32
urea (mmol/L)	6.39±0.60	5.48±0.58	6.15±1.14	4.94±0.63*

Table 1. Summary of hematology and biochemistry data (Mean ± SD). *, $p<0.05$ compared with the control group.

Histological images show the osteogenic capability of different treatments (Fig 2). Eight weeks after implantation, we observed that the empty defects were filled by fibrous tissue and they cannot heal spontaneously, although very little newly formed bone was seen along the host bone. This confirmed that the bone defects were critical-sized. In the defects filled with β-TCP, we observed that some new bone predominantly deposited on the surface of the bone substitute closest to the host bone, whereas the bone substitute particles were encircled by fibrous tissue in the center of the defects. In contrast, when BMP-2 incorporated β-TCP was implanted, the defects were filled by irregular cavities of bone marrow, interspersed bioceramic particles and interconnected trabecular and woven bone, which was osteoconductive bone on the periphery of the defects in substantial contact with the host bone and osteoinductive bone in the center of the defects. Although the bone trabecula was suspended by fibrous tissue in the defects filled with autologous bone, a mass of new bone can be found in the surrounding area of both the host bone bed and the residual bone autograft. In summary, defects filled with BMP-2 incorporated β-TCP and autologous bone demonstrated a

similar performance regarding both osteoconductive and osteoinductive bone regeneration.

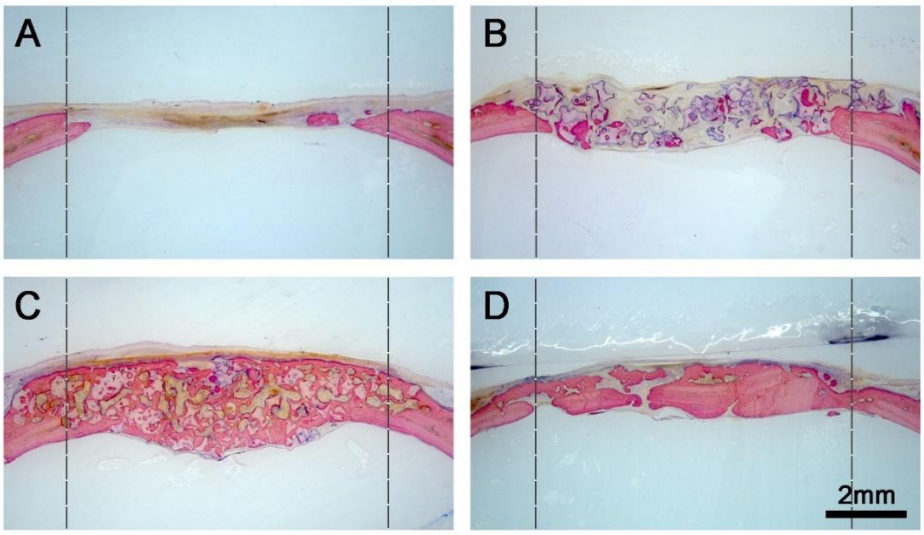

Figure 2. Representative histological images of each group (A: empty defects (control), B: defects filled with β-TCP, C: defects filled with BMP-2 incorporated β-TCP, D: defects filled with autologous bone) after eight weeks' implantation. The dotted lines indicate the original boundary of defects.

The detailed measurements of defect filling, including bone volume, bone density, fibrous tissue density, and residual material density, were shown in Figure 3. It revealed that the new bone volume was significantly higher when the defects were filled with BMP-2 incorporated β-TCP compared with all the other treatments. However, when the defects were filled with the BMP-2 incorporated β-TCP and autologous bone, the bone density was similar. This might be caused by a large amount of marrow cavity in the BMP-2 incorporated β-TCP. The fibrous tissue density in the BMP-2 incorporated β-TCP group was significantly less compared with all the other groups. On the other hand, the residual material density showed no difference between the β-TCP group and the BMP-2 incorporated β-TCP group. In summary, the BMP-2 incorporated β-TCP is comparable with autologous bone in its osteogenic capability of repairing critical-sized bone defects.

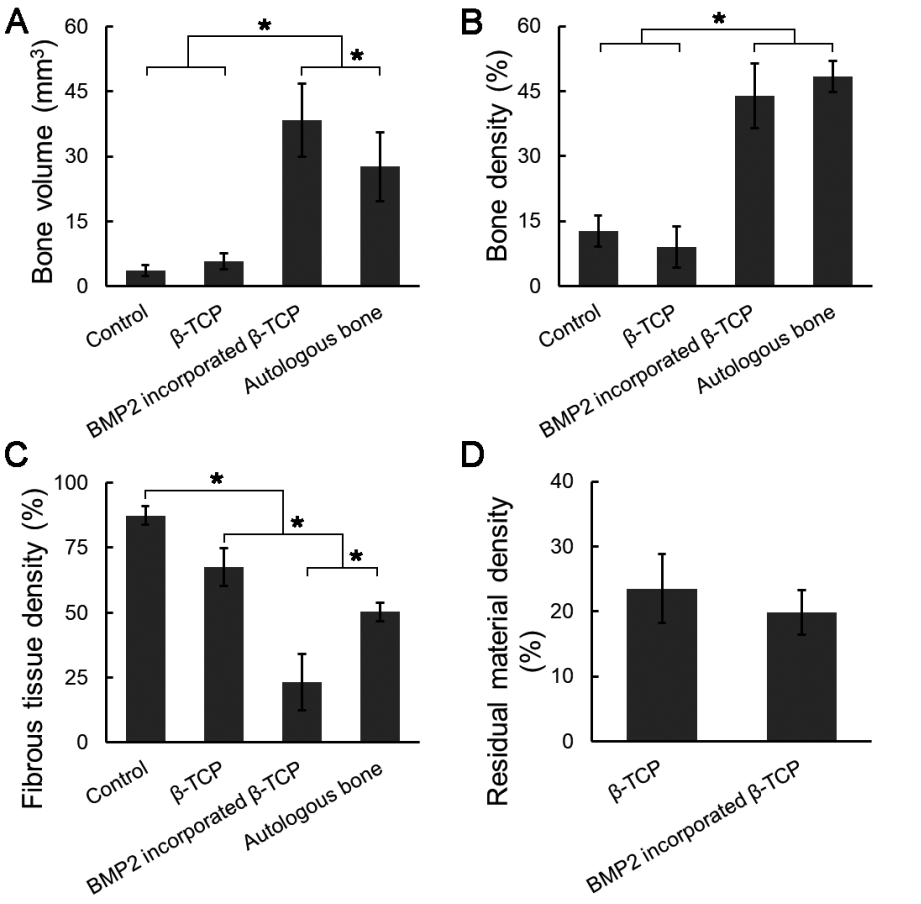

Figure 3. Histomorphometric measurements of bone volume (A), bone density (B), fibrous tissue density (C) and residual material density (D). Values are shown as the median ± standard deviation. *P < 0.05.

Summary

The results from the present study demonstrate that BMP-2 incorporated β-TCP could be a promising substitute for autologous bone used for bone regeneration. Future clinical trials and preclinical trials with large animal models are necessary to investigate the safety and efficacy of BMP-2 incorporated β-TCP.

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